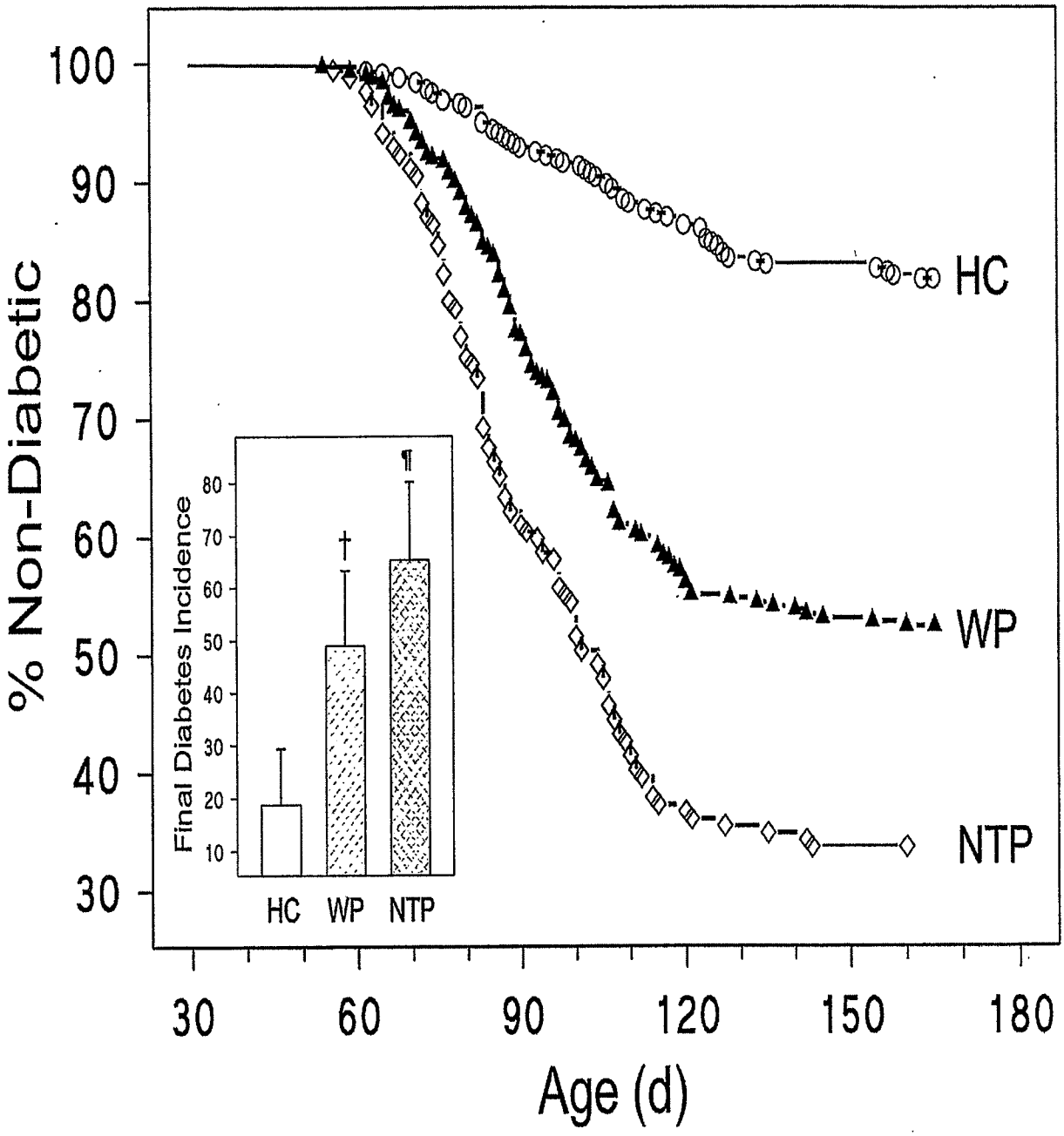

100
90
80
70
60
50
40
30
% Non-Diabetic
30
60
90
120
150
180
Age (d)
HC
WP
NTP
Final Diabetes Incidence
80
70
60
50
40
30
20
10
†
¶
HC
WP
NTP

Figure 1

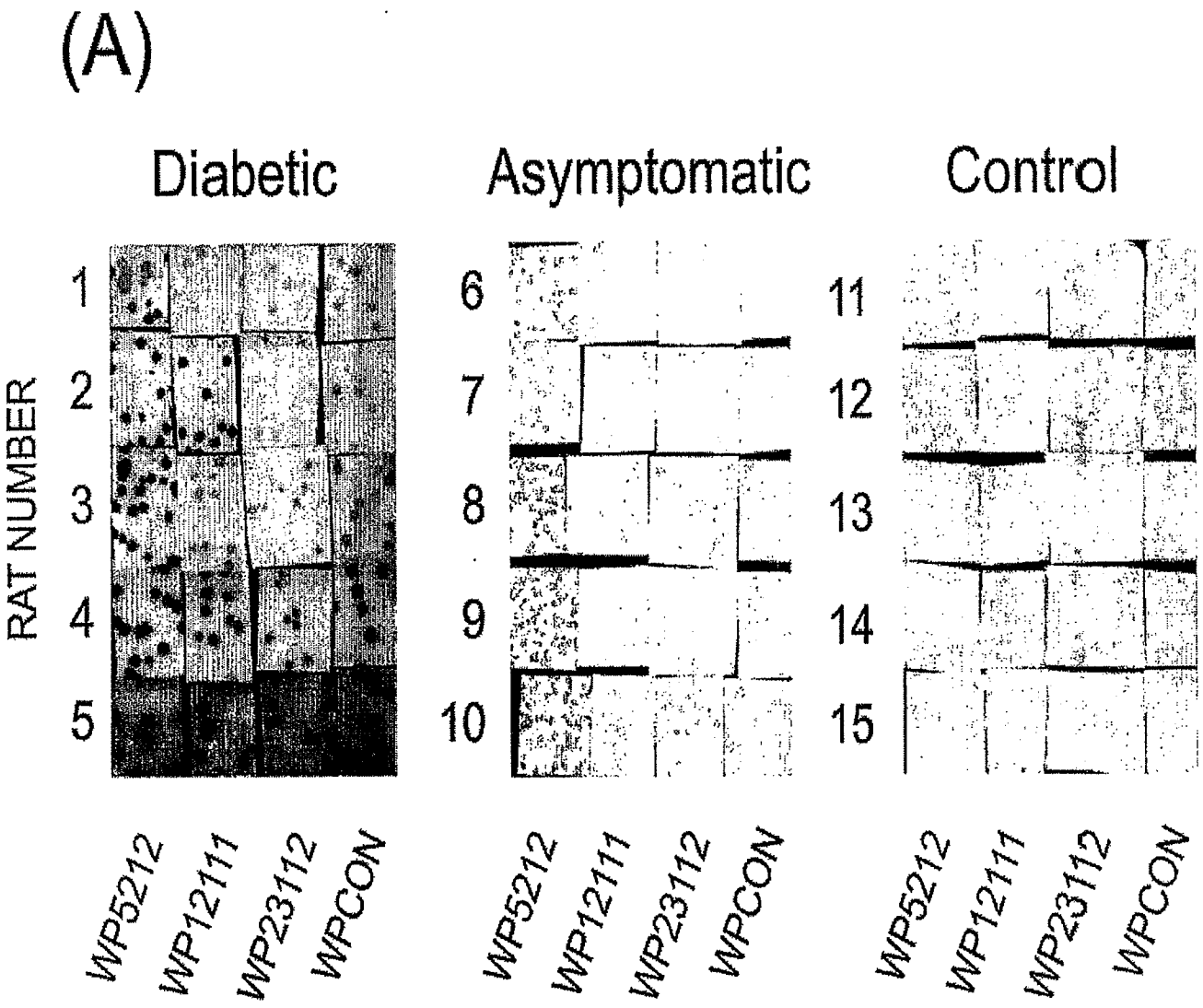

(A)
Diabetic
Asymptomatic
Control
RAT NUMBER
1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
WP5212
WP12111
WP23112
WPCON
WP5212
WP12111
WP23112
WPCON
WP5212
WP12111
WP23112
WPCON

Figure 2A

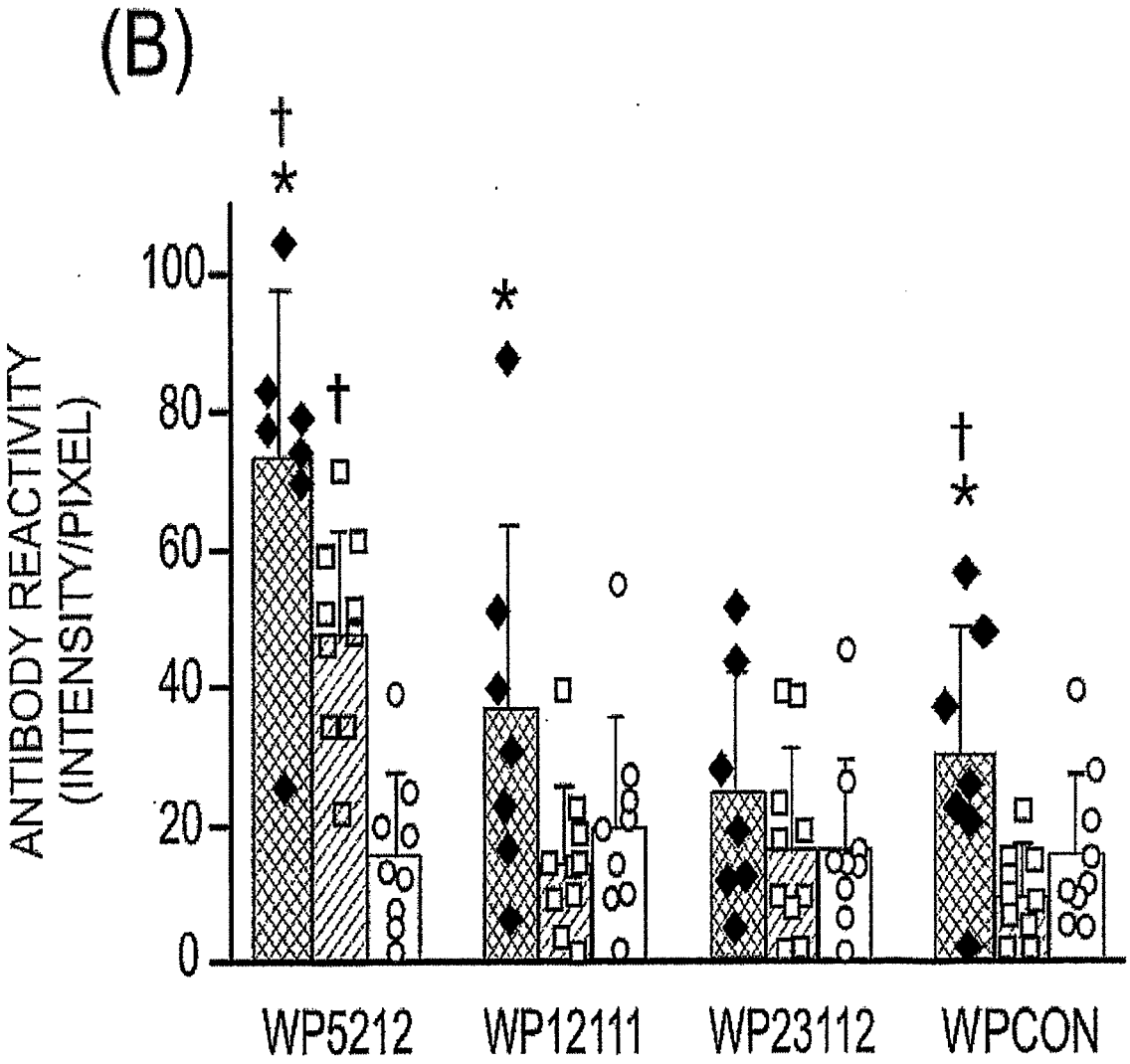

(B)
ANTIBODY REACTIVITY
(INTENSITY/PIXEL)
100
80
60
40
20
0
WP5212
WP12111
WP23112
WPCON

Figure 2B

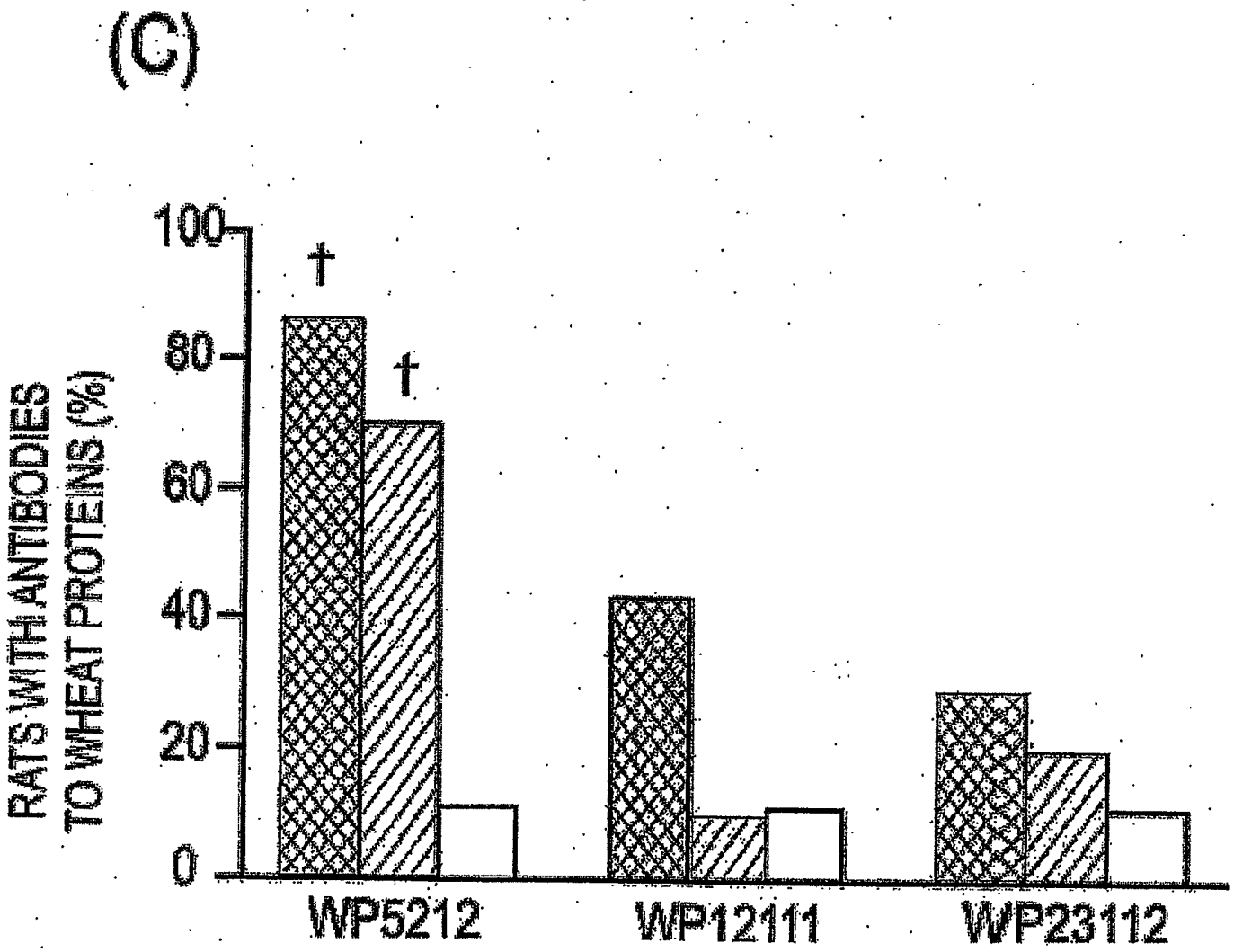

(C)
RATS WITH ANTIBODIES TO WHEAT PROTEINS (%)
100
80
60
40
20
0
†
†
WP5212
WP12111
WP23112

Figure 2C

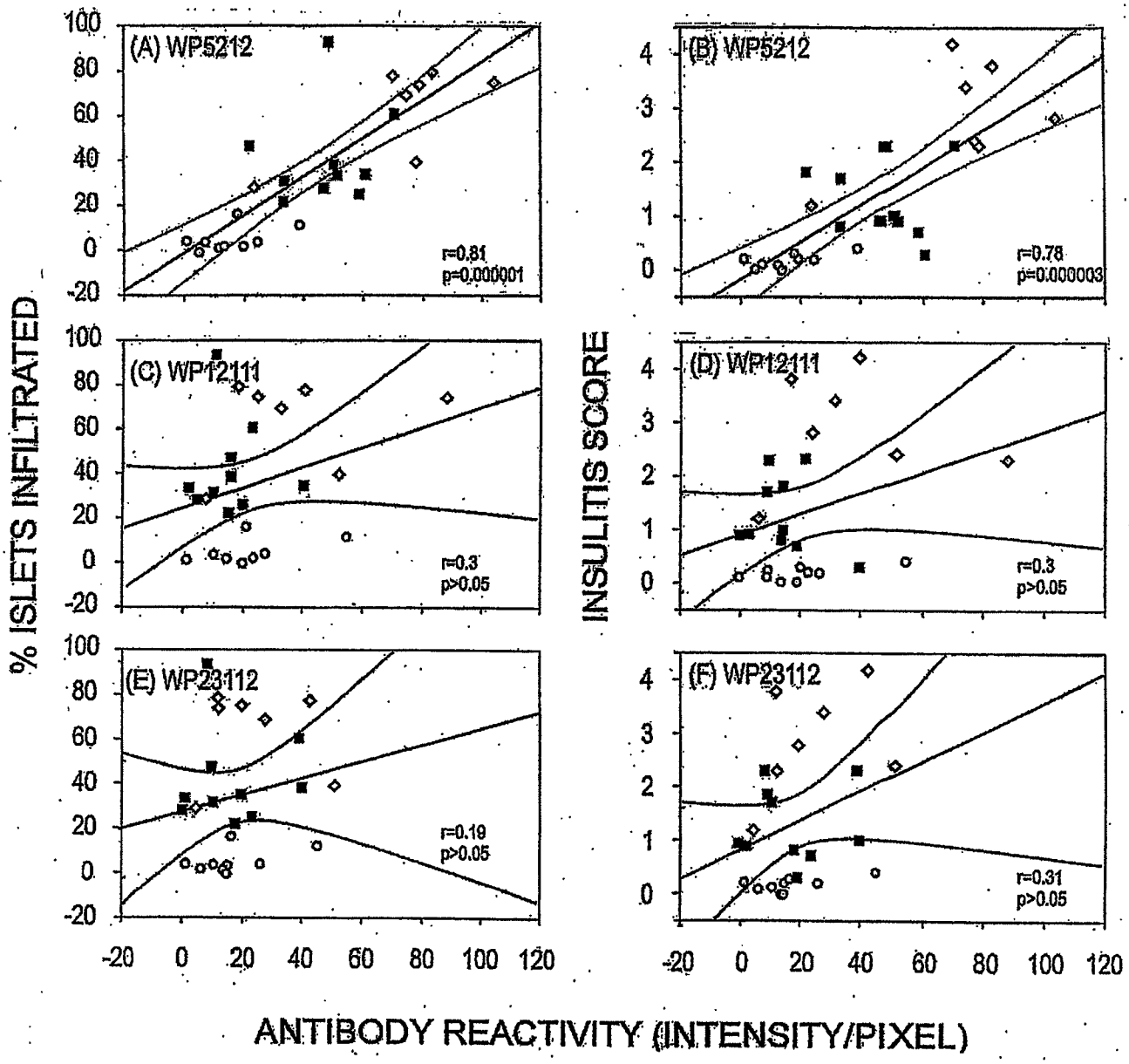

(A) WP5212
r=0.81
p=0.000001
(B) WP5212
r=0.78
p=0.000003
(C) WP12111
r=0.3
p>0.05
(D) WP12111
r=0.3
p>0.05
(E) WP23112
r=0.19
p>0.05
(F) WP23112
r=0.31
p>0.05
% ISLETS INFILTRATED
INSULITIS SCORE
ANTIBODY REACTIVITY (INTENSITY/PIXEL)

Figure 3

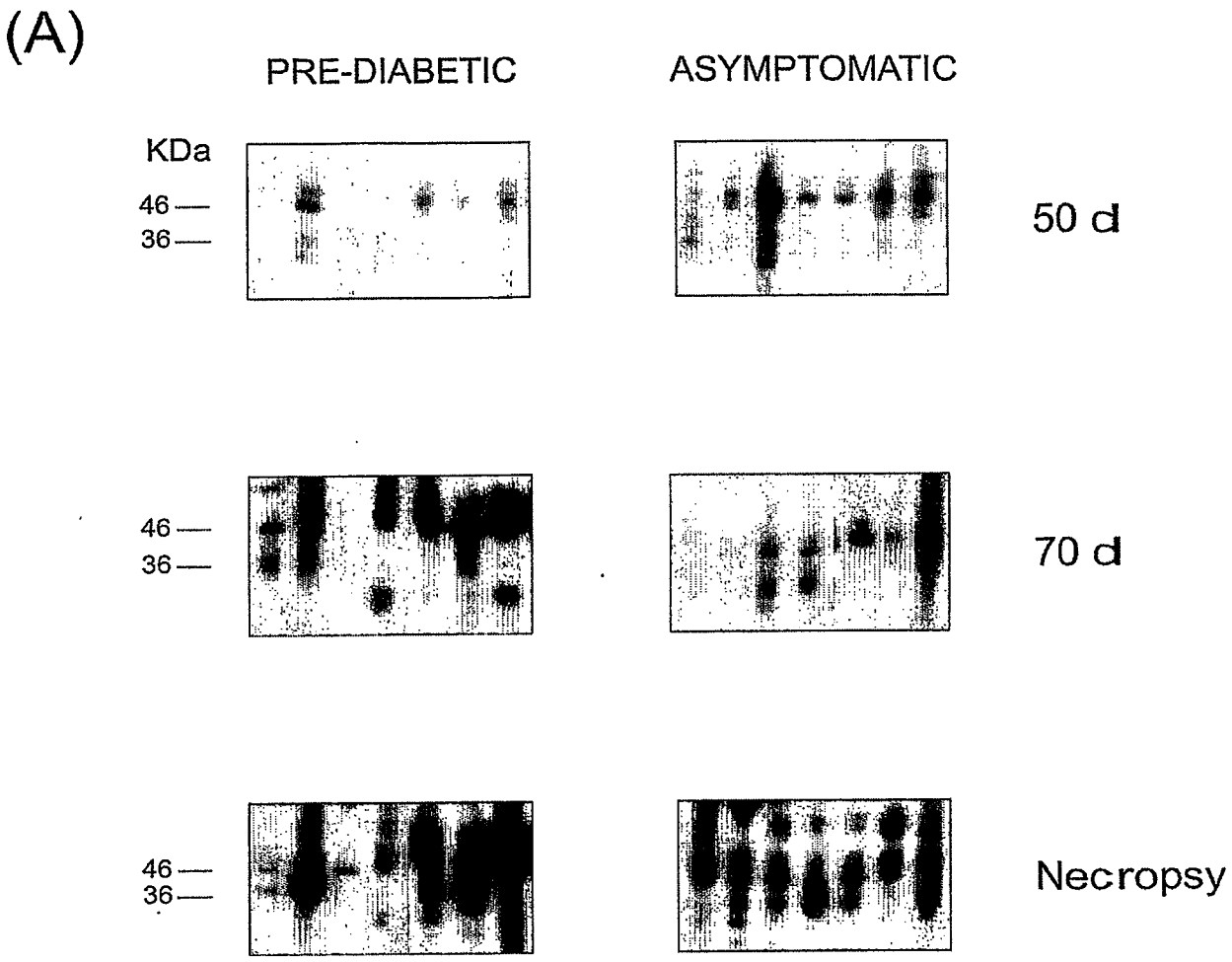

(A)
PRE-DIABETIC
ASYMPTOMATIC
KDa
46
36
50 d
70 d
Necropsy
(B)
70 d
Necropsy
INTENSITY (x 10² Pixels)
25
20
15
10
5
0
†
‡
58 54 52 50 46 40 38 36 33 29
MOLECULAR WEIGHT (kDa)

Figure 4

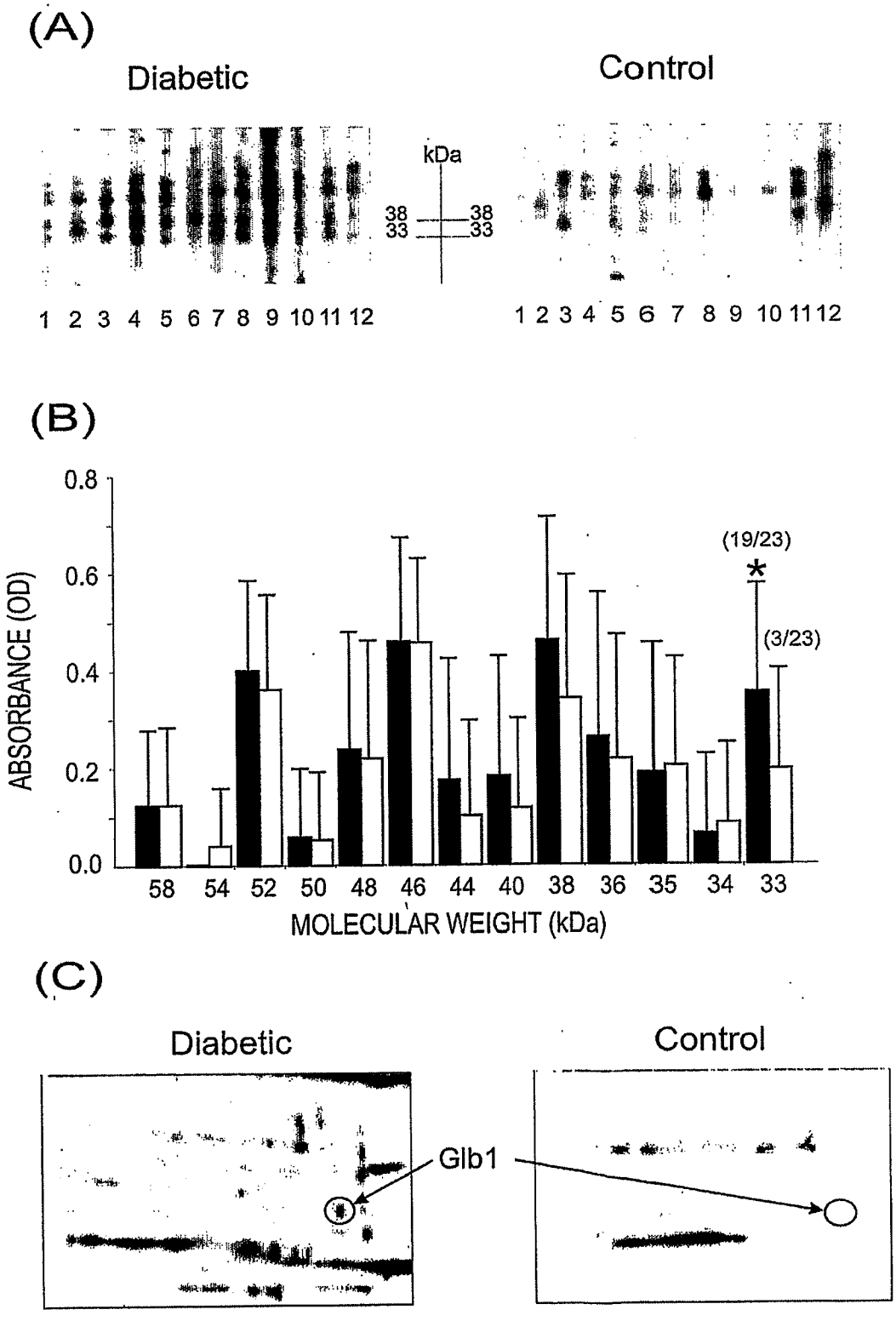
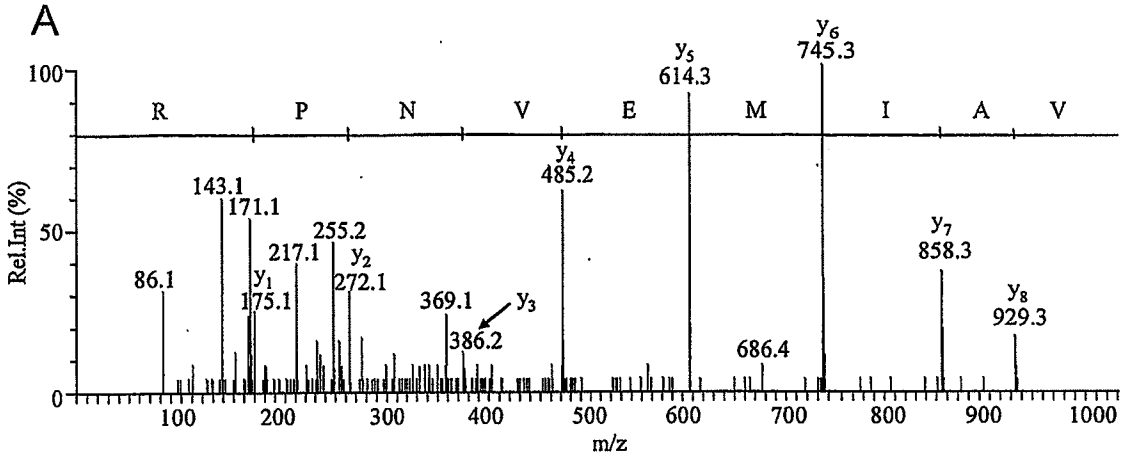

(A)
Diabetic
Control
kDa
38
33
38
33
1 2 3 4 5 6 7 8 9 10 11 12
1 2 3 4 5 6 7 8 9 10 11 12
(B)
ABSORBANCE (OD)
0.0
0.2
0.4
0.6
0.8
(19/23)
*
(3/23)
58 54 52 50 48 46 44 40 38 36 35 34 33
MOLECULAR WEIGHT (kDa)
(C)
Diabetic
Control
Glb1

Figure 5

A



B

PANEL B Theoretical and observed tryptic peptides of WSG and WP5212

Theoretical fragmentation [a] of Glb1 (Acc. No. AAA34269)	**Theoretical fragmentation [a] of WP5212**	**Experimental fragmentation**
RPYVFGPR	**RPYVFGPR**	**RPYVFGPR**
VAIMEVNPR	**VAIMEVNPR**	**VAIMEVNPR**
AQDQDEGFVAGPEQQSR	ATIPLLFLLGTSLLFAAAVSASHDEEEDR	
FQFLSVKPLLASLSK	AFVVPGLTDADGVGYVAQGEGVLTVIENGEK	
GSESESEEEEEQQR	VAVANITPGSMTAPYLNTQSFK	
LGSPAQELTFGRPAR	QGDVIVAPAGSIMHLANTDGR	
DTFNLLEQRPK	LAVVLEGEGEVEIVCPHLGR	
SFHALANQDVR	GSAFVVPPGHPVVEIASSR	
GGHSLQQCVQR	DQQDEGFVAGPEQQQEHER	
ALRPFDQVSR	QASEGDQGHHWPLPPFR	
IIQSDHGFVR	GSSNLQVVCFEINAER	
HEQEEEQGR	LDDPAQELAFGRPAR	
GDEAVETFLR	FQYFSAKPLLASLSK	
EQEQEQER	GSGSESEEEQDQQR	
ILHTISVPGK	DTFNLLEQRPK	
EEEEDDQR	SFHALAQHDVR	
EAAEGGQGHR	GDEAVEAFLR	
DDQQQHGR	ALRPFDEVSR	
	ILHTISVPGK	
	GDSSTMATR	
	SEEEEDDR	
	DDQQQHGR	
	HEQEEQGR	
	DEEHGDGR	
	LGSLLGSR	
	LYEADAR	

[a] Theoretical fragmentation was calculated using the PeptideCutterprogram (Swiss Prot (2002) website address: http://us.expasy.org/tools/peptidecutter, Swiss Institute of Bioinformatics, Epalinges, Switzerland)

Figure 6

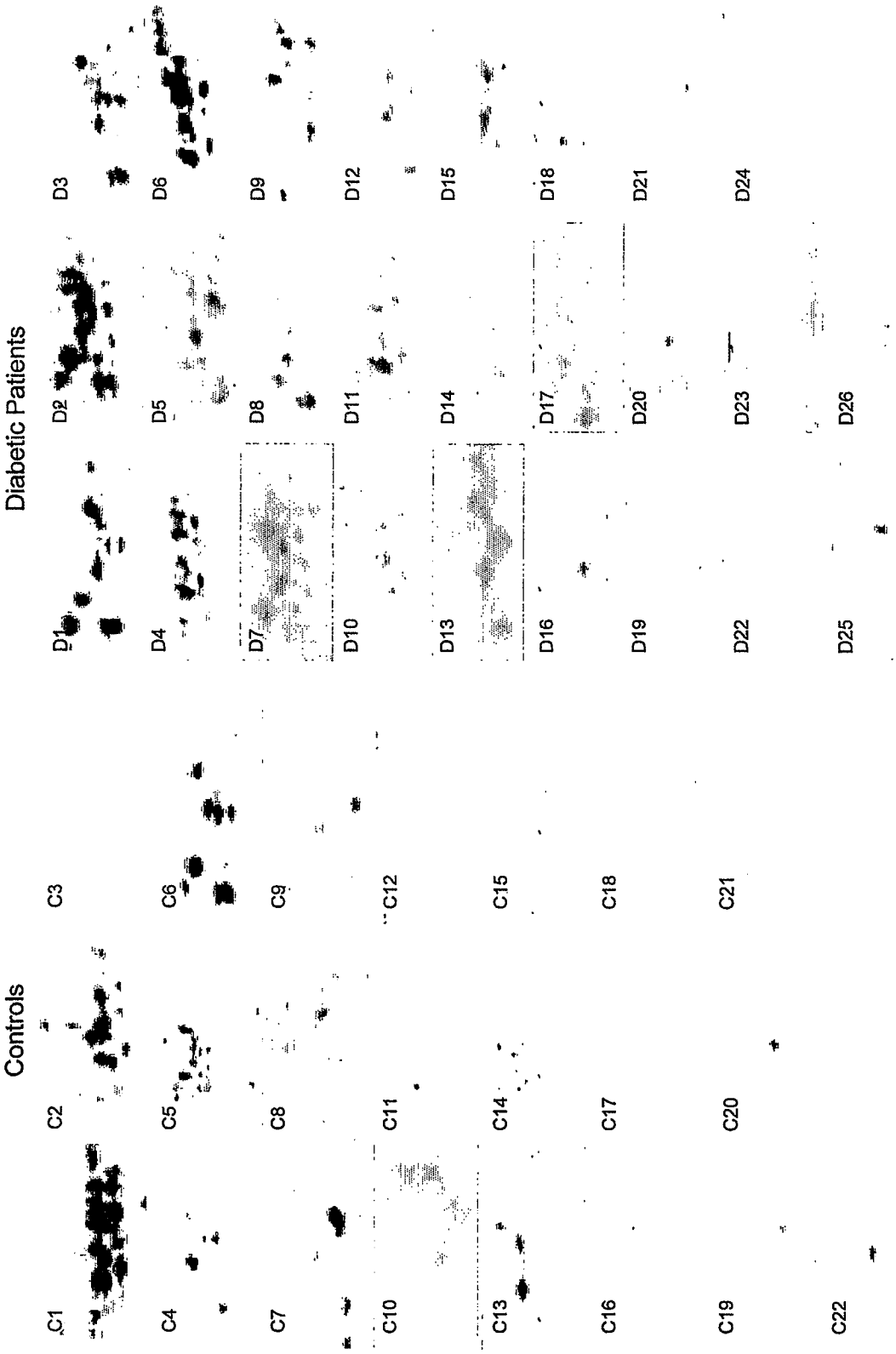

Controls
Diabetic Patients
C1
C2
C3
C4
C5
C6
C7
C8
C9
C10
C11
C12
C13
C14
C15
C16
C17
C18
C19
C20
C21
C22
D1
D2
D3
D4
D5
D6
D7
D8
D9
D10
D11
D12
D13
D14
D15
D16
D17
D18
D19
D20
D21
D22
D23
D24
D25
D26

Figure 7

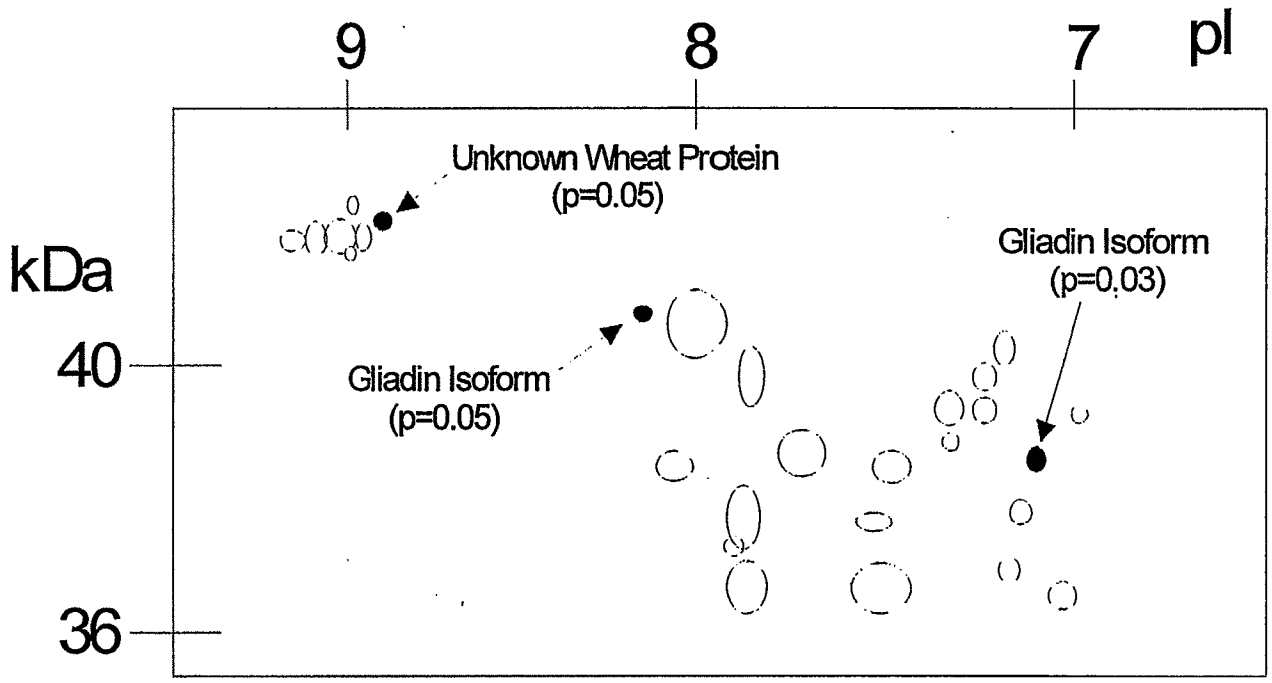
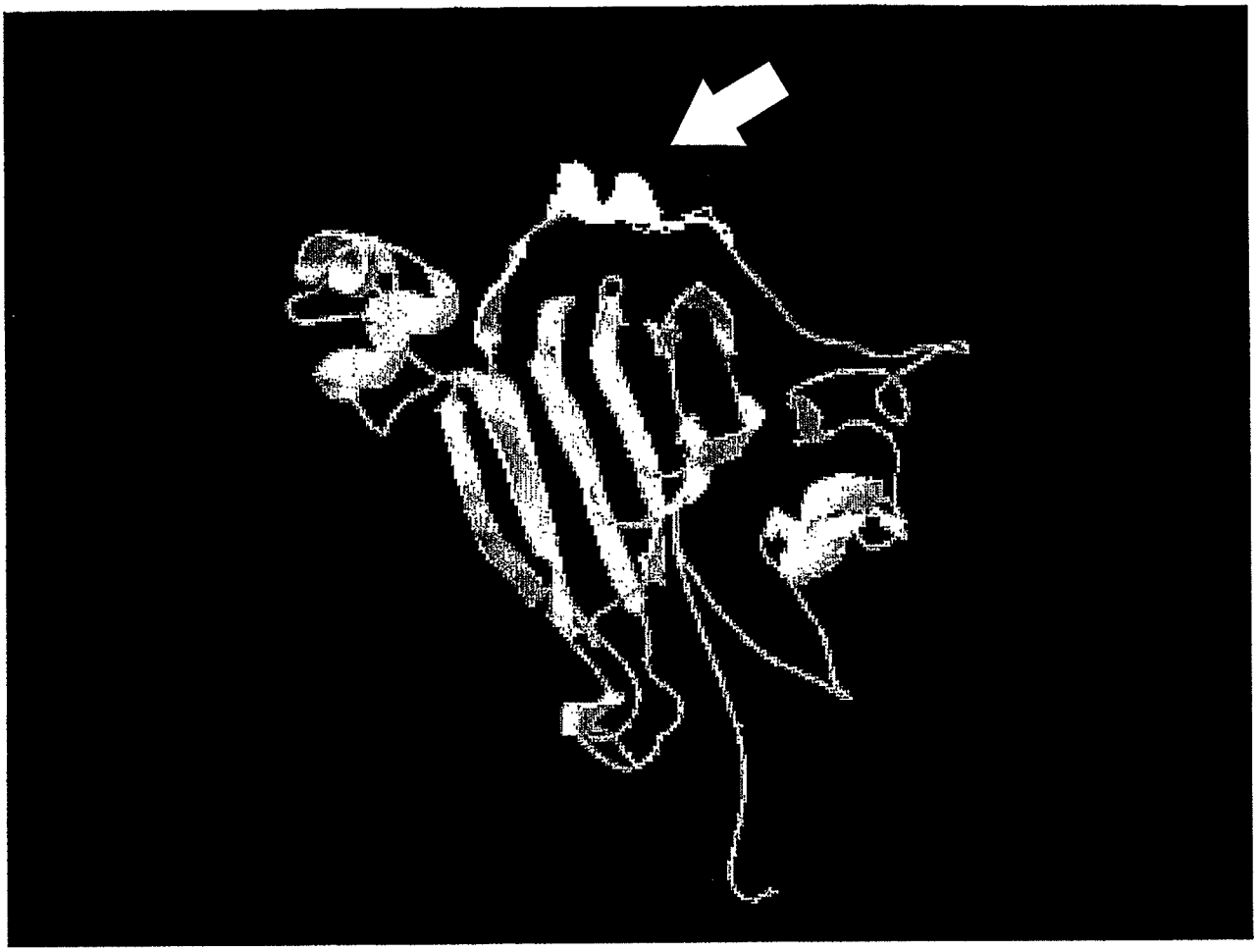

9
8
7
pI
Unknown Wheat Protein
(p=0.05)
Gliadin Isoform
(p=0.03)
kDa
40
Gliadin Isoform
(p=0.05)
36

Figure 8

Figure 9



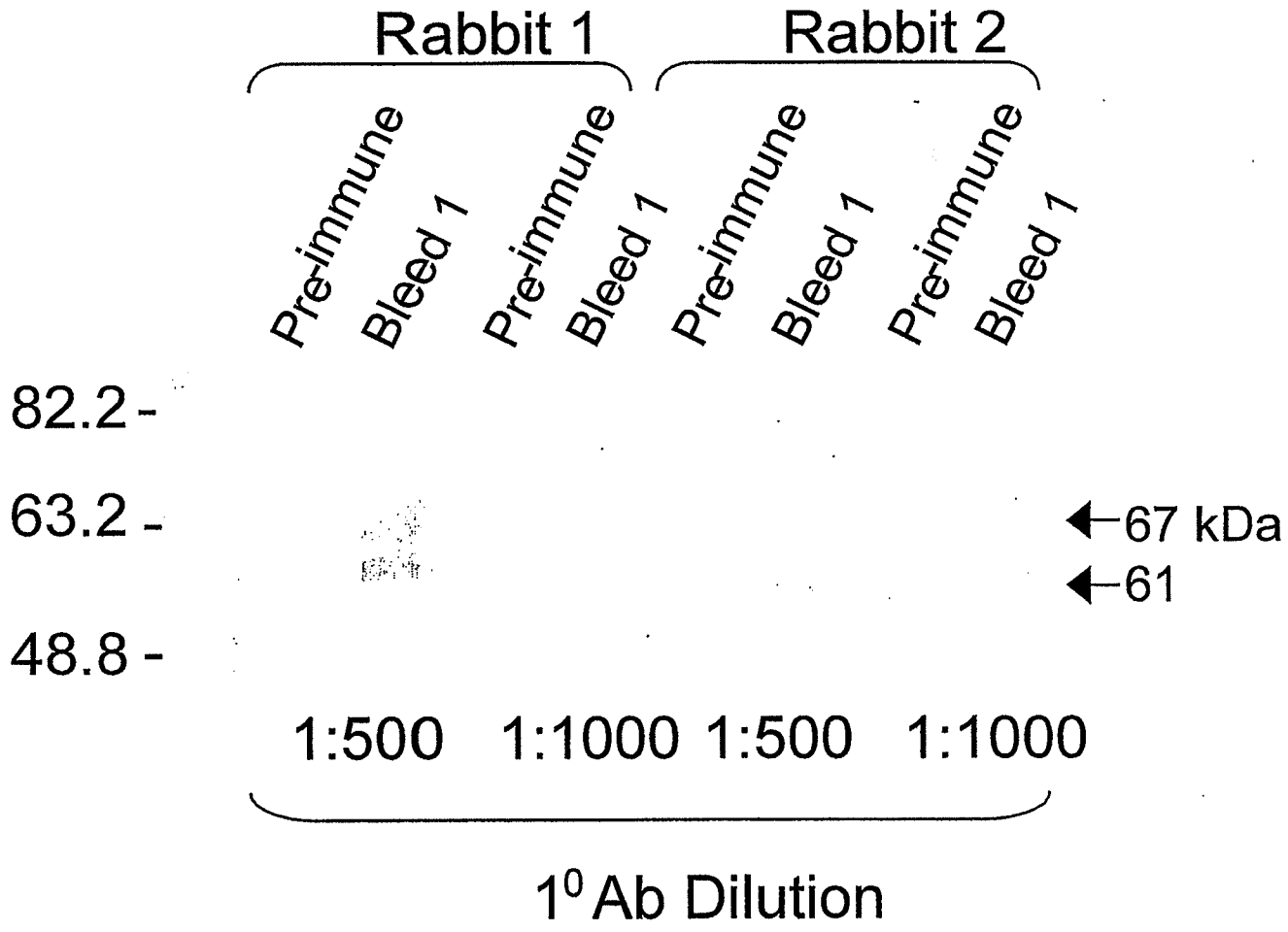

Rabbit 1
Rabbit 2
Pre-immune
Bleed 1
Pre-immune
Bleed 1
Pre-immune
Bleed 1
Pre-immune
Bleed 1
82.2 -
63.2 -
48.8 -
67 kDa
61
1:500
1:1000
1:500
1:1000
1⁰ Ab Dilution

Figure 11

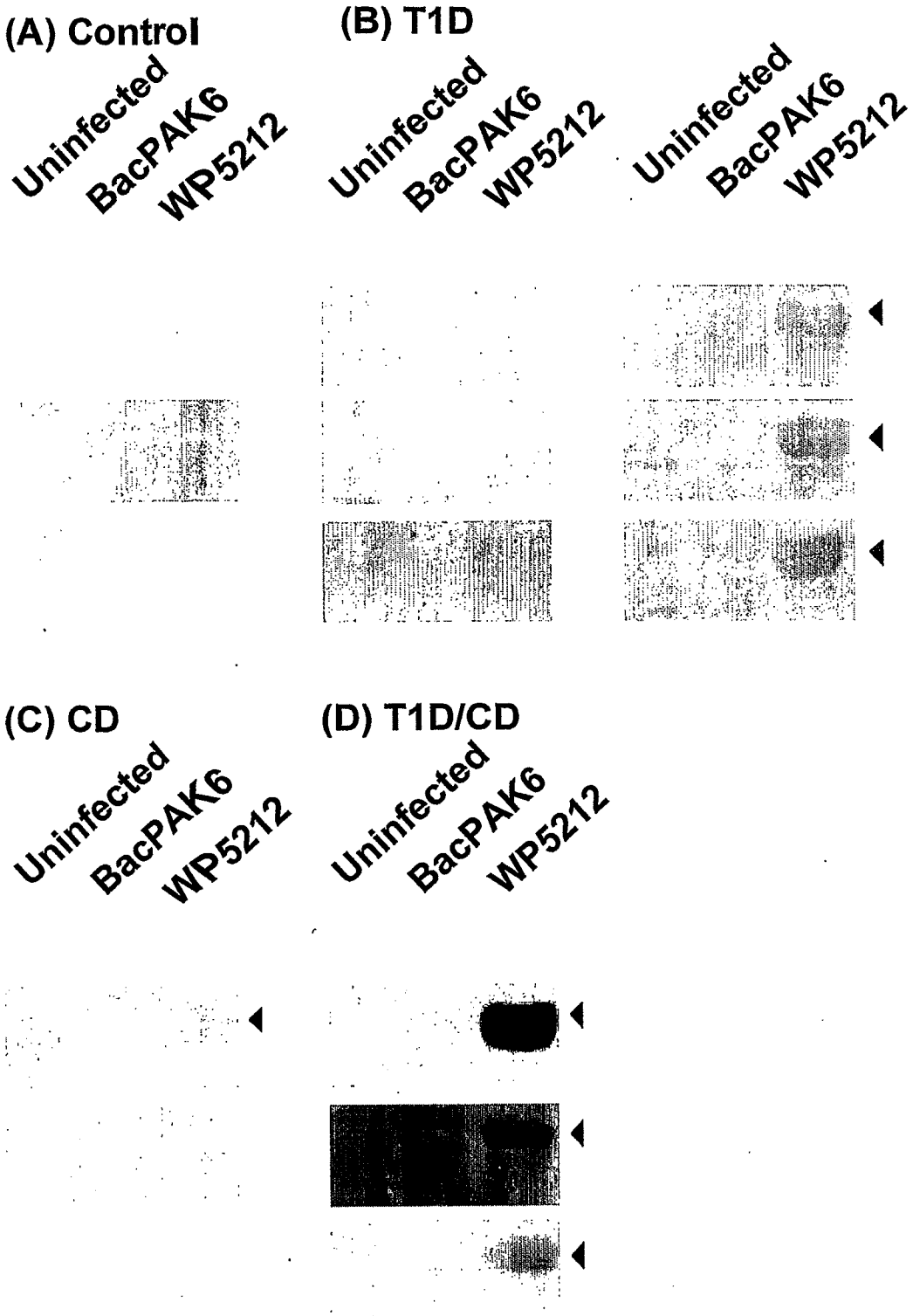

(A) Control
Uninfected
BacPAK6
WP5212
(B) T1D
Uninfected
BacPAK6
WP5212
Uninfected
BacPAK6
WP5212
(C) CD
Uninfected
BacPAK6
WP5212
(D) T1D/CD
Uninfected
BacPAK6
WP5212

Figure 12A

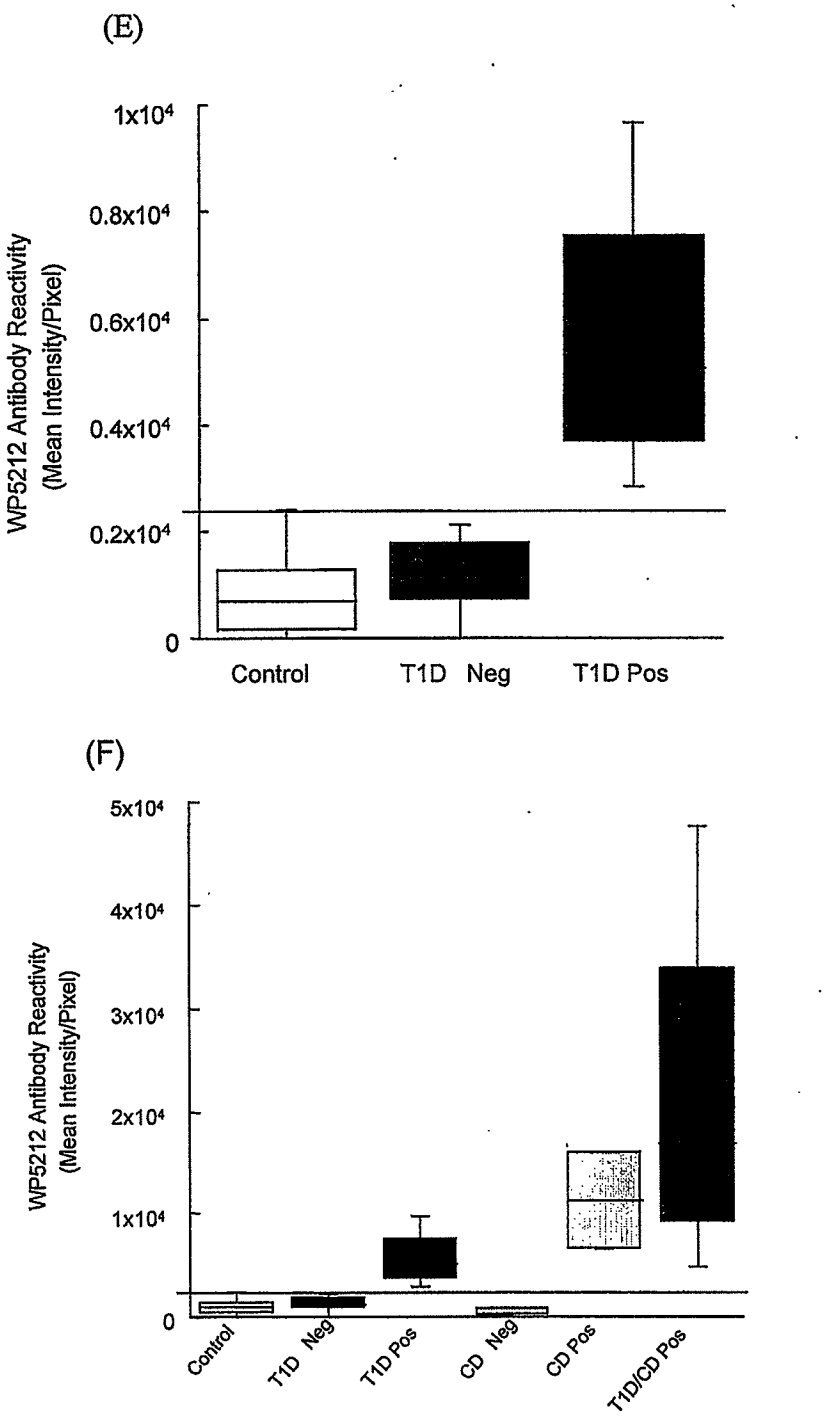

(E)
1x10⁴
0.8x10⁴
0.6x10⁴
0.4x10⁴
0.2x10⁴
0
WP5212 Antibody Reactivity
(Mean Intensity/Pixel)
Control
T1D Neg
T1D Pos
(F)
5x10⁴
4x10⁴
3x10⁴
2x10⁴
1x10⁴
0
WP5212 Antibody Reactivity
(Mean Intensity/Pixel)
Control
T1D Neg
T1D Pos
CD Neg
CD Pos
T1D/CD Pos

Figure 12B

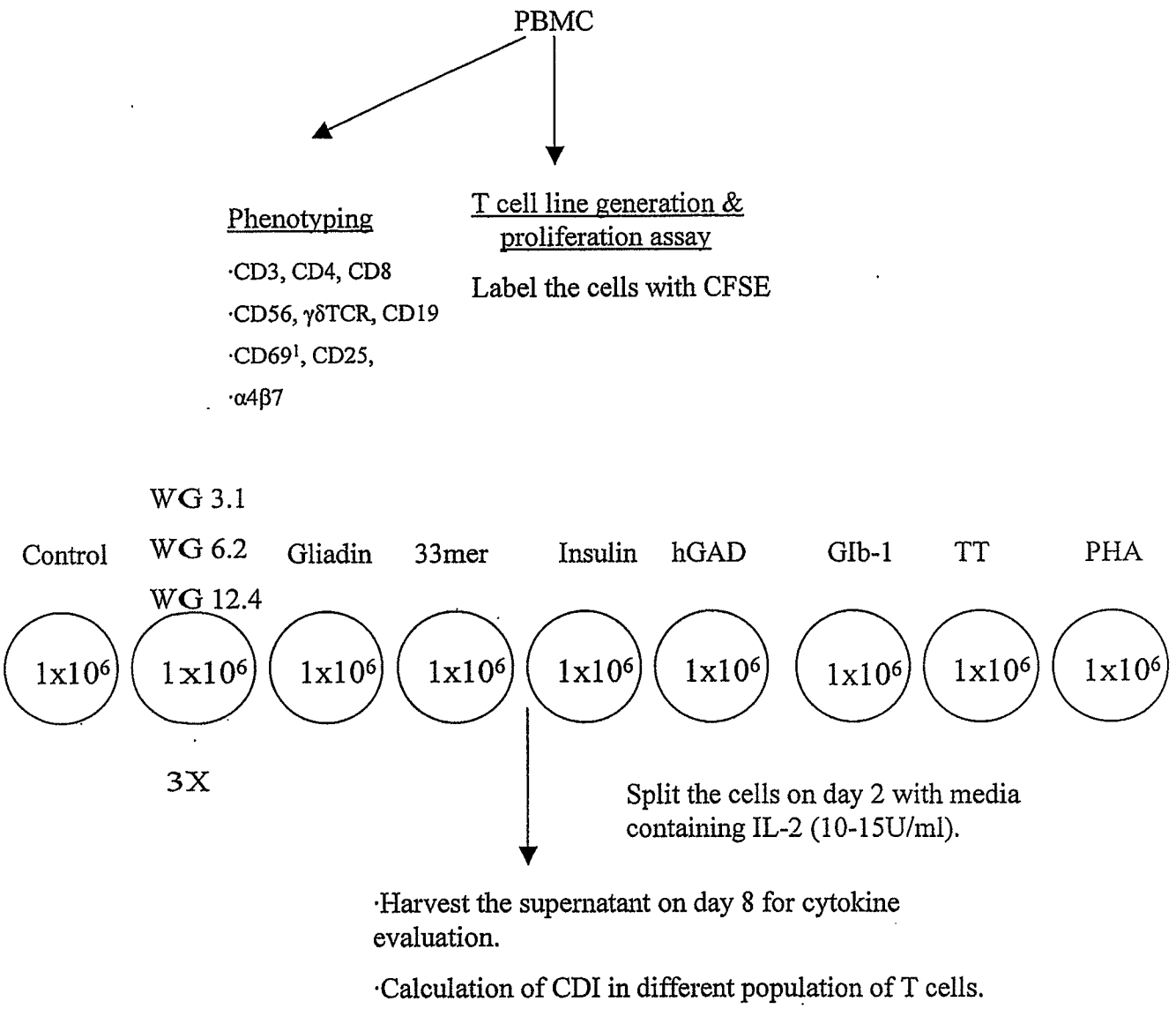
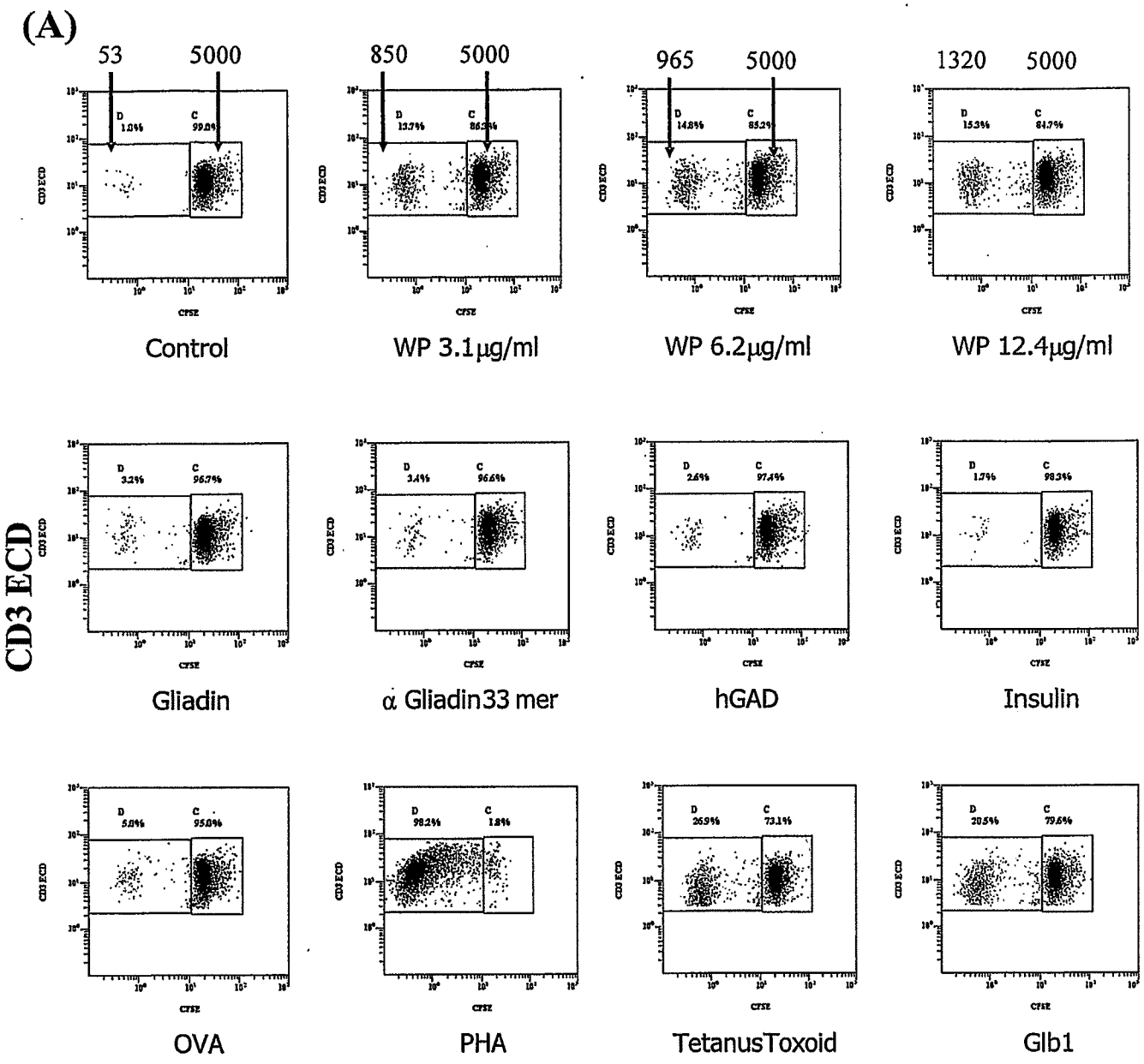

PBMC
Phenotyping
·CD3, CD4, CD8
·CD56, γδTCR, CD19
·CD69[1], CD25,
·α4β7
T cell line generation & proliferation assay
Label the cells with CFSE
WG 3.1
WG 6.2
WG 12.4
Control
Gliadin
33mer
Insulin
hGAD
GIb-1
TT
PHA
1x10^6
3X
Split the cells on day 2 with media containing IL-2 (10-15U/ml).
·Harvest the supernatant on day 8 for cytokine evaluation.
·Calculation of CDI in different population of T cells.

Figure 13



B

$$\text{CDI} : \frac{\text{Number of CD3}^{+}\text{, CFSE}^{\text{dim}}\text{ cells with antigen}}{\text{Number of CD3}^{+}\text{, CFSE}^{\text{dim}}\text{ cells without antigen}}$$

Figure 14

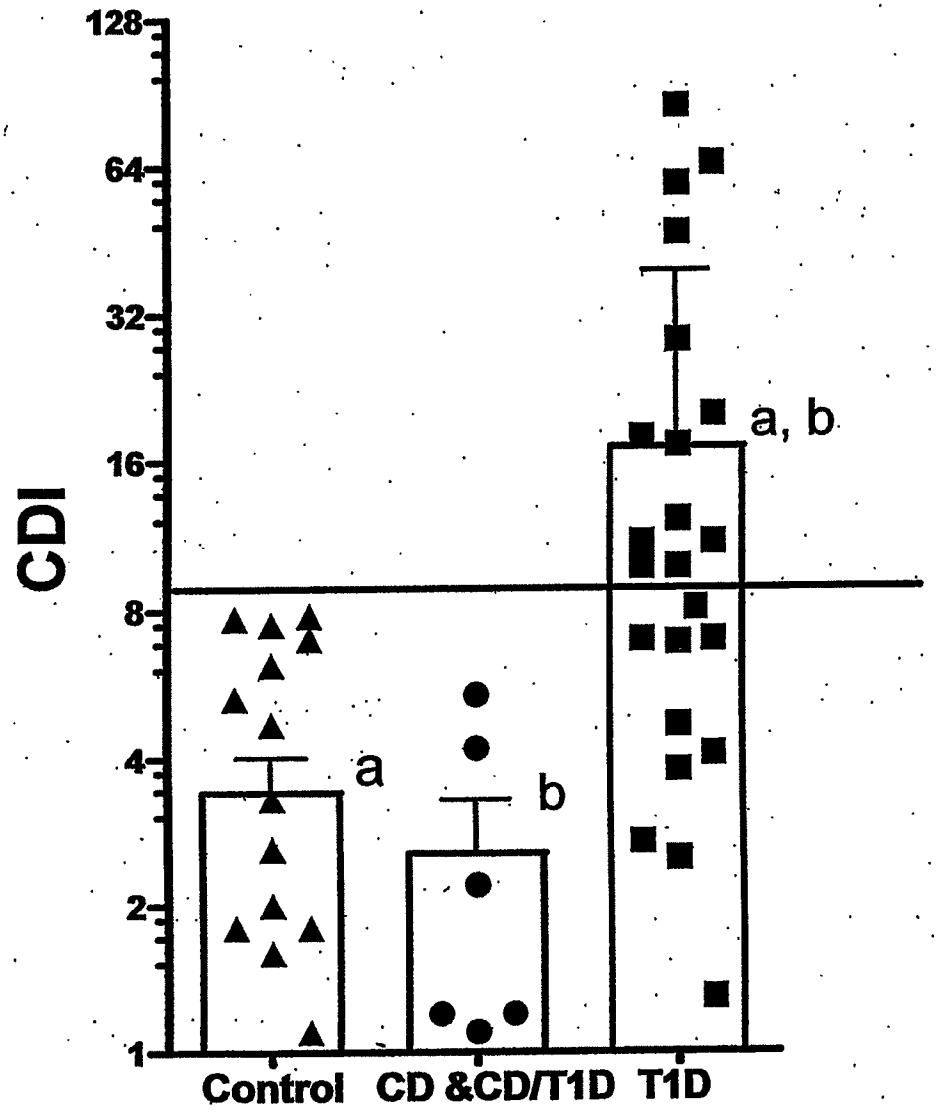


Figure 15

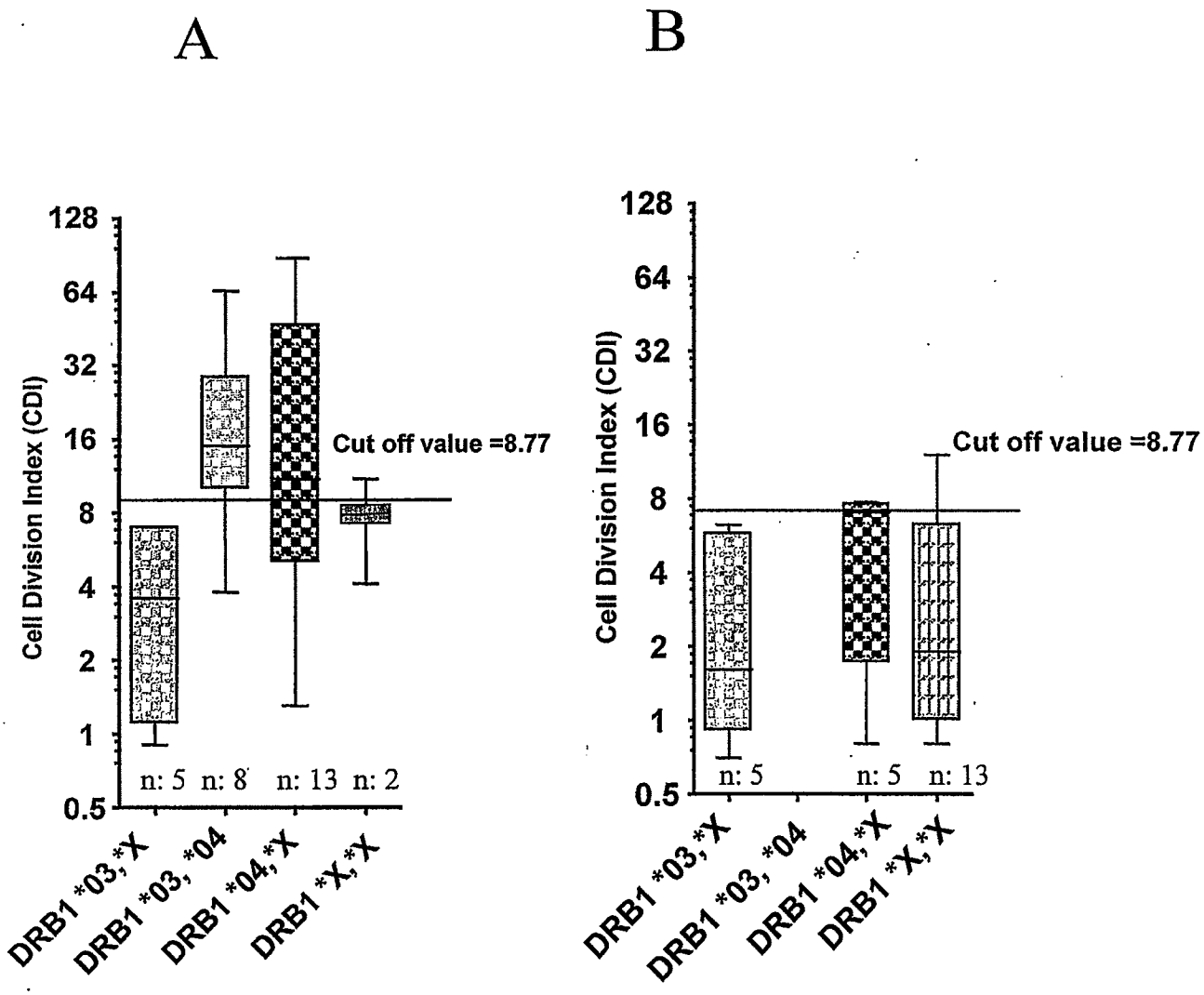

A
B
128
64
32
16
8
4
2
1
0.5
Cell Division Index (CDI)
Cut off value =8.77
n: 5
n: 8
n: 13
n: 2
n: 5
n: 5
n: 13
DRB1 *03,*X
DRB1 *03, *04
DRB1 *04,*X
DRB1 *X,*X

Figure 16